REMARKS

Claims 1-13 and 15-21 were pending in this application. According to the October 23, 2002 Office Action, claims 1-13 and 15-21 were rejected. Applicant has canceled claims 1-13 and 15-21 and added new claims 22-35. Accordingly, claims 22-35 are under consideration. Applicant maintains that the amendments do not introduce any new matter.

A Submission of Formal Drawings is also being filed herewith.

Specifically, claim 22 is supported by original claim 1 and the specification. For instance, the term "a process for producing a transgenic plant, whose seeds have an increased amount of reserve material in comparison with a wild-type plant" is supported by claim 1. The terms "the expression of an endogenous invertase inhibitor protein" is "reduced" or "eliminated" "during the development of seeds" is supported in the specification on page 3, lines 14-16. The term "the activity of the invertase, which is subject to a regulation by the invertase inhibitor protein" is increased during the development of seeds is supported in the specification on page 5, lines 5-7 and 25-27. The term "an increased accumulation of reserve material in the seed" is supported in the specification on from page 3, lines 20-24. Furthermore, the term "Obtaining a nucleotide sequence ... from flowers with young ovules of a plant" is supported in original claim 1. The idea of having the coding nucleotide sequence inserted in a DNA construct in sense orientation or antisense orientation next to a promoter is supported in original claim 7 and on page 7, lines 10-12. The term "Transforming a plant cell of said plant, from which the coding nucleotide sequence is obtained, with a DNA construct" and "cultivating of the plant cell and regenerating of a plant" is supported in original claim 1.

New claim 23 is supported by original claim 14 and the specification. For instance, the term "separating and purifying an inhibitor protein fraction from the cell wall protein fraction" is supported in the specification on page 8, line 20 to page 9, line 21. The term "digesting of the inhibitor protein and separating of the resulting peptides" is supported in the specification on page 9, lines 23-28. The term "sequencing of the peptides in order to obtain the amino acid sequences" is supported in the specification on page 9, lines 29-32. The term "derivating nucleotide sequences from the amino acid sequence and designing of primers" is supported in the

specification on page 9, last paragraph. Finally, the term "cloning a partial or full-length cDNA ..." is supported by original claim 14.

New claims 24 and 25 correspond to original claims 3 and 4. New claim 26 is based on original claim 5 and supported by the specification on page 3, last line.

Rejection under 35 U.S.C. §112, First Paragraph

The Examiner rejected claims 1-13 and 15-21 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. The Examiner also rejected claims 1-13 and 17-21 under 35 U.S.C. §112, first paragraph, allegedly because the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

In response, Applicant respectfully traverses the Examiner's rejection. The Examiner argues that the specification does not teach isolation of any invertase coding sequences other than a tobacco invertase inhibitor sequence nor does it teach alteration of seed development in any plant species other than tobacco. Applicant respectfully disagrees.

The present application provides a teaching according to which a transgenic plant, whose seeds have an increased amount of reserve material, can be produced by the inhibition of the expression of a specific endogenous invertase inhibitor (which is active during the development of seeds). By the inhibition of this specific invertase inhibitor, the activity of that invertase which is only active during seed development and which is subject to a regulation by said invertase inhibitor will be increased resulting in an increased accumulation of reserve material. According to the present application, the inhibition of the expression of the invertase inhibitor is achieved by the introduction of a nucleotide sequence coding for the invertase inhibitor protein to be inhibited.

Although the application provides only one example (namely the use of the apoplastic tobacco invertase inhibitor gene for the production of transgenic tobacco plants) which only

illustrates the practicability of the present procedure, the present procedure can be used for alteration of seed development in any plant species other than tobacco. As evidence for the foregoing, Applicant submits herewith a Declaration under Rule 132 showing that the invertase inhibitor isoform (Bn-inhh1) from rape can be used for the transformation of rape plants whereby transgenic rape plants are obtained whose seed have an increased content of oily compounds. The Declaration in question shows the results of these experiments which were carried out according to the teachings of the specification. Furthermore, this Declaration shows the nucleic acid sequence coding for Bn-inhh1 (rape invertase inhibitor) and the corresponding amino acid sequence of this invertase inhibitor (in comparison to the amino acid sequence of the tobacco invertase inhibitor).

These results show that the present procedure can be used also for other plant species and not only for tobacco.

The Examiner refers to problems connected with the isolation of autologous DNA sequences and with the recombinant change of metabolic processes. Specifically, concerning the documents cited by the Examiner, Applicant has the following remarks:

Broun et al., PNAS 98:8925-8927 (2001)

According to the Examiner, an important consideration in genetic engineering for altered starch or protein content would be to know whether the gene is under some kind of regulatory control or influenced by additional mechanisms governing metabolic flux.

On page 8926, second column, second paragraph, of Broun et al., it is described that the overexpression of rate-limiting enzymes may not result in enhanced flux through the pathway since catalysis might be downregulated by feedback inhibition. As an example, a feedback-insensitive regulatory enzyme of the lysine biosynthetic pathway is described, which was overexpressed in transgenic plants. Although flux through the pathway was increased, increases in lysine accumulation were limited by enhanced breakdown. Furthermore, a feedback-insensitive regulatory enzyme of the fatty acid biosynthesis pathway is described, which was overexpressed in seeds of transgenic canola plants. Here a relatively slight increase in seed oil was obtained (about 5%).

In view of these statement, the results obtained in the present invention with the tobacco invertase inhibitor and with the rape invertase inhibitor are rather surprising. Table 2 of the description shows that the seed oil content of transgenic tobacco plant exceeds that of wild-type plants by 20%-70%, whereas the protein content of the seeds of transgenic tobacco plants exceeds that of wild-type plants by 5-34%. Also, the seed oil content of transgenic rape plants is much higher than that of wild-type rape (type Drakkar; see enclosed Declaration).

Despite the Examiner's assertions, the statements found in the document of Broun et al. are not relevant to the present application. The increase of the reserve material content, according to the present application, is not achieved by an overexpression of a protein which regulates a biosynthetic pathway (as described in Broun et al.), but rather by the inhibition of such a protein. By the antisense inhibition of the endogenous invertase inhibitor the activity of the invertase which is regulated by the invertase inhibitor is increased. Thereby, the maximum activity of the naturally occurring cell wall invertase will be by no means exceeded. Only the activity phase of the cell wall invertase will be extended (see page 7, line 25, page 8, line 19). In this context, it is noteworthy that the natural cell wall invertases expressed during the seed development are naturally already strongly expressed.

Broun et al., Science, 282 (1998)

According to the Examiner the isolation of orthologous DNA sequences from other species would introduce an element of unpredictability. This limitation would be introduced in finding homologous regions that would adequately enable either PCR amplification or Southern hybridization and would entail using either degenerate primers or probes with limited homology. The Examiner refers to the documents of Broun et al., which would provide an example, that already a small number of changes to the coding region would result in an enzyme with completely changed activity.

In this document, it is described on page 1315, left column, first paragraph, that an oleate hydroxylase exhibits a high degree of sequence similarity to oleate desaturases. Both enzymes catalyse similar reactions; oleate desaturases catalyse the O_2-dependent insertion of a double bond between carbons 12 and 13 of lipid-linked oleic acid whereby linoleic acid is produced.

Oleate hydroxylases catalyse the generation of the structurally related hydroxy fatty acid, ricinoleic acid. This means that both enzymes are closely related.

This document is by no means relevant to the present teaching since the present procedure does not require the use of degenerate primers, nor the use of DNA probes with limited homology. This results from the following:

1) Several publications already describe the isolation of several invertase inhibitors from different plant species (for example maize, potato, etc.). This means that already several invertase inhibitors are available which could be used for the present procedure.

2) New claim 23 describes that the invertase inhibitor gene used for the present method can be identified by the isolation of the corresponding protein and the subsequent determination of the amino acid sequences. This means, in order to obtain an invertase inhibitor gene which can be used for the present method, it is not necessary to use PCR amplification or Southern hybridization and to use degenerate primers or probes with limited homology. The present application provides an alternative method to obtain usable inhibitor genes.

3) In the present procedure, invertase inhibitor genes are used which are obtained from a cDNA library of flowers with young ovules. In such a cDNA library, already cDNAs are strongly accumulated that code for invertase inhibitor which is expressed predominantly during the development of seeds.

4) The family of invertase inhibitor genes shows, in contrast to other gene families, only a limited sequence homology. The apoplastic invertase inhibitors of closely related plant species (such as Arabidopsis/rape; tobacco/tomato/potato; Vivia faba/Glycine max) have on the protein level a maximum sequence identity of approximately 80%. However, the sequence identity between these plant families is only about 40% (for example sequence identity between Arabidopsis and tobacco). Furthermore, the invertase inhibitors have only a very low homology to for example the inhibitors of pectin methyl esterases, whereby for example, the sequence identity between the invertase inhibitor of Arabibopsis and the pectin methyl esterase inhibitor of Arabidopsis is only 20%.

In view of the above, it can be concluded that a chance to isolate many genes other than those of interest is in case of the invertase inhibitor practically negligent.

Elomaa et al., Molecular Breeding, 2:41-50 (1996)

According to the Examiner, the phenotypic character expected from expression of a DNA construct often cannot be reliably predicted, in particular, in view of the likely presence of isoforms of the target gene.

In the abstract of this document, it is described that the transformation of Gerbera with gchs1 and gchs2 in antisense orientation led to partial or complete inhibition of the resident sense gene expression in most of the transformants. In addition, the expression of other members of the gene family were differentially affected in the transformants (see page 42, left column, second paragraph). The degree of inhibition was influenced by the overall sequence homology between the members studied (see page 48, left column). On page 49, left column, first paragraph, it is stated that the data described shows, that to ensure suppression of a particular member of the gene family, gene specific antisense transgenes must be designed.

The Applicant notes that the above phenomenon was taken into consideration in the present invention. The transgenic plants of the present invention are gene specific antisense transgenes. According to the present application, a nucleotide sequence coding for that invertase inhibitor to be inhibited is introduced in a plant in order to increase the activity of the invertase expressed during the development of seeds.

Büssis et al., Planta, 202:126-136 (1997)

According to the Examiner, this document would show the unpredictability in metabolic engineering. The document of Büssis et al. describes potato plants expressing a heterologous yeast invertase directed either to the apoplast, vacuole or cytosol.

The differences between the document of Büssis et al. and the present application are as follows:

1) In contrast to the present application, the document of Büssis et al. does not describe a homologous transformation but a heterologous transformation.

2) Another difference is, that in the present invention, the transformation of a plant has a very specific effect, since the invertase inhibitor to be blocked will be only expressed at a certain time in a very defined tissue of the plant. In the document of Büssis et al., however, a constitutive overexpression of an invertase throughout the whole plant is described. This means that the modification described by Büssis et al. is very unspecific. Therefore, many undesired side effects occur.

Therefore, the document of Büssis et al. is by no means relevant for the present teaching.

Accordingly, in view of the above remarks and the Declaration, the Examiner is kindly requested to withdraw this rejection.

Rejection under 35 U.S.C. §112, Second Paragraph

The Examiner rejected claims 1-13 and 15-21 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

In response, Applicant has canceled claims 1-13 and 15-21 and added new claims 22-35 which did away with objected language. Accordingly, the Examiner is kindly requested to withdraw this rejection.

Rejection Under 35 U.S.C. §101

The Examiner rejected claims 16-17 under 35 U.S.C. §101 allegedly because the claimed invention is directed to non-statutory subject matter.

In response, Applicant has canceled claims 16 and 17 and thus, the Examiner is kindly requested to withdraw this rejection

Rejection under 35 U.S.C. §102(b)

The Examiner rejected claims 1-3, 5-8, 10, 12-13 and 15-21 under 35 U.S.C. §102(b) as allegedly being anticipated by Rausch (WO 98/04722) which corresponds to U.S. Patent 6,384,300.

In response, Applicant respectfully traverses this rejection. Rausch relates to and teaches a nucleic acid coding for an invertase inhibitor. This invertase inhibitor reduces the enzymatic activity of an invertase. Furthermore, Rausch teaches processes for the production of transgenic plants, which are characterized by a reduction of those sucrose losses, which normally occur during storage. Storage sucrose losses occur *inter alia* in the potato tuber (cold sweetening of potatoes), in the beet of sugar beet and in the fruits of the tomato plant.

The differences between the present procedure and the teachings in Rausch are as follows:

1) Whereas Rausch teaches the reduction of storage sucrose losses in specific storage organs (such as the beet of sugar beets, the tuber of potato and the fruit of tomato plants), the present application relates to a process for the production of plants whose seeds have an increased amount of reserve material. Therefore, by using the presently claimed procedure, different parts or organs of plants are altered in comparison to those plants described in Rausch.

2) The development of storage organs is regulated by completely different regulatory mechanisms than the development of seeds. Furthermore, a reserve material of seeds as defined by the present application is for example starch, fat and protein (see page 6, line 13), but not sucrose. Such reserve material is formed in different biosynthetic pathways than sucrose. Therefore, by using the presently claimed procedure, different regulatory mechanisms within the plants are altered when compared to Rausch.

3) The present application describes transgenic plants that have already before storage an increased reserve material content in comparison to wild-type plants, whereas the transgenic plants described in Rausch have an increased sucrose content in comparison to wild-type plants after storage.

4) In the presently claimed procedure, the expression of the invertase inhibitor which is expressed in young seeds during the flower stage will be inhibited (by the use of that nucleotide sequence coding for said invertase inhibitor) which results in an increased activity of the invertase. In contrast to the present invention, Rausch teaches that an invertase inhibitor will be overexposed so that the activity of the invertase will be decreased.

5) The invertase inhibitor used in the present invention is different from the invertase inhibitor used in the document cited.

Accordingly, the present invention could not be anticipated by the cited prior art and the Examiner is kindly requested to withdraw this rejection.

Rejection under 35 U.S.C. §103

The Examiner rejected claims 1-13 and 15-21 under 35 U.S.C. §103(a) as allegedly unpatentable over Rausch in view of Gordon-Kamm.

In response, Applicant respectfully traverses this rejection. Applicant reiterates the remarks made about Rausch hereinabove. Importantly Rausch is directed to a different subject matter and as indicated above, it does not teach or suggest the reduction or elimination of the expression of the invertase inhibitor which is specifically expressed in certain flower stages in the ovule by the introduction of a gene coding for the invertase inhibitor to be inhibited. Also, Rausch does not teach or suggest the inhibition of the expression of the invertase inhibitor in order to increase the activity of that invertase which is normally expressed during the seed development. Furthermore, Rausch does not teach or suggest that the inhibition of the invertase inhibitor expression leads to an increased accumulation of reserve materials (starch, fat, protein) within the seeds of a plant.

Gordon-Kamm teaches the transformation of maize with a construct comprising the CaMV35S promoter and NOS terminator and therefore does not supplement the teachings of Rausch to render the present invention obvious. Specifically, Gordon-Kamm does not teach to reduce or eliminate the expression of the invertase inhibitor which is specifically expressed in certain flower stages in the ovule by the introduction of a gene coding for the invertase inhibitor

to be inhibited. Also, Gordon-Kamm et al. does not teach to inhibit the expression of the invertase inhibitor in order to increase the activity of that invertase which is normally expressed during the seed development. Furthermore, Gordon-Kamm et al. does not teach that the inhibition of the invertase inhibitor expression leads to an increased accumulation of reserve materials within the seeds of a plant.

In summary, the combined teachings of Rausch and Gordon-Kamm provide no motivation for the person skilled in the art to inhibit the expression of the invertase inhibitor during the development of seeds in order to increase the accumulation of reserve material. Accordingly, the Examiner is kindly requested to withdraw this rejection.

Double Patenting

The Examiner rejected claims 15-17 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-22 of U.S. Patent No. 6,384,300 (Rausch '300).

In response, Applicant has hereinabove canceled claims 15-17 and thus, the Examiner is kindly requested to withdraw this rejection.

In light of the foregoing, it is respectfully submitted that this application is now in condition to be allowed and the early issuance of a Notice of Allowance is respectfully solicited. If there are any issues or amendments the Examiner wishes to discuss, the Examiner is encouraged to contact the undersigned.

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on February 19, 2003:

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APPENDIX A
"Clean" Version of Each Paragraph/Section/Claim
37 C.F.R. § 1.121(b)(ii) and (c)(i)

CLAIMS (with indication of amended or new):

22. (NEW) A process for producing a transgenic plant, whose seeds have an increased amount of reserve material in comparison with a wild-type plant due to the reduction or elimination of the expression of an endogenous invertase inhibitor protein during the development of seeds so that the activity of invertase, which is subject to a regulation by the invertase inhibitor protein, is increased during the development of seeds leading to an increased accumulation of reserve material in the seed, said process comprising the steps of:

(a) obtaining a nucleotide sequence coding for the invertase inhibitor protein from a cDNA library from flowers with young ovules of a plant;

(b) inserting the coding nucleotide sequence in a DNA construct in sense or anti-sense orientation next to a promoter as a regulatory unit;

(c) transforming a plant cell of said plant, from which the coding nucleotide sequence was obtained, with the DNA construct; and

(d) cultivating the plant cell and regenerating a plant, wherein the expression of the endogenous invertase inhibitor protein is reduced or eliminated during seed development.

23. (NEW) Process according to claim 22, wherein the nucleotide sequence coding for an invertase inhibitor protein is a cDNA, obtained by the following steps:

(a) separating and purifying an inhibitor protein fraction from the cell wall protein fraction of flowers with young ovules of a plant;

(b) digesting the inhibitor protein and separation of the resulting peptides;

(c) sequencing the peptides in order to obtain the amino acid sequences;

(d) deriving nucleotide sequences from the amino acid sequences and designing of primers; and

(e) cloning a partial or full-length cDNA coding the invertase inhibitor protein from a cDNA library from flowers with young ovules of said plant or alternatively synthesizing the partial or full-length cDNA using the primers.

24. (NEW) A process according to claim 22, in which the promoter is a constitutive or inducible promoter.

25. (NEW) A process according to claim 24, in which the promoter is selected from the group consisting of CaMV35S promoter, ubiquitin promoter, and zein promoter from corn.

26. (NEW) A process according to claim 22 in which the nucleotide sequence coding for the invertase inhibitor is a nucleotide sequence having a sequence identity of 80% or more to the cDNA sequence in the cDNA library from flowers with young ovules of a plant .

27. (NEW) A process according to claim 22, in which the invertase inhibitor is an apoplastic invertase inhibitor.

28. (NEW) A process according to claim 22, in which the DNA construct has additional regulatory units.

29. (NEW) A process according to claim 28, in which an additional regulatory unit is a transcription termination signal.

30. (NEW) A process according to claim 29, in which the transcription termination signal comes from a NOS gene of *Agrobacterium tumefaciens*.

31. (NEW) A process according to claim 22, in which the plant cell is a cell of a dicotyledonous or monocotyledonous plant.

32. (NEW) A process according to claim 31, in which the plant cell is from a plant selected from the group consisting of rape, sunflower, peanut, soy bean, oil palm, rice, corn, wheat, barley, oats, rye, pea, Calendula officinalis, Coriandrum sativum, Crambe abyssinica, Cuphea ssp., Dimorphotheca pluvialis, Euphorbia lagascae, Euphorbia lathyris, Lesquerella grandiflora, Limnanthes alba, Linum usitatissimum, Lunaria annua, Lunaria biennis, Oenothera ssp., Ricinus communis and Simmondsia chinensis.

33. (NEW) A process according to claim 22, in which the DNA construct is in a vector.

34. (NEW) A process according to claim 33, in which the vector is a plasmid or a virus.

35. (NEW) A process according to claim 22, in which the transformation of the plant cell is carried out by an Agrobacterium tumefaciens-mediated transformation or a biolytic process comprising a step selected from the group consisting of electrically induced DNA absorption, chemically induced DNA absorption, electroporation, macroinjection, microinjection and PEG-mediated transformation.

Cl Cont.